

MODIFIED VENOM AND VENOM COMPONENTS AS ANTI-RETROVIRAL AGENTS

BACKGROUND OF THE INVENTION

1. Field of the Invention:

The present invention relates to a class of proteins, a process of production thereof, and a method for treatment of neurological and viral diseases and especially to the treatment of heretofore intractable diseases such as retro-viral infections, including specifically HIV infections.

2. Description of Prior Art:

Sanders, et al. had commenced investigating the application of modified venoms to the treatment of ALS in 1953 having employed poliomyelitis infection in monkeys as a model. Others antiviral studies had reported inhibition of pseudorabies (a herpesvirus) and Semliki Forest virus (alpha-virus). See Sanders' U.S. Patent Nos. 3,888,977, 4,126,676, and 4,162,303. Sanders justified the pursuit of this line of research through reference to the studies of Lamb and Hunter (1904) though it is believed that the original idea was postulated by Haast. See Haast U.S. Patent Nos. 4,341,762 and 4,741,902. See also MacDonald, et al., U.S. Patent No. 5,723,477. The studies of Lamb and Hunter (Lancet 1:20, 1904) showed by histopathologic experiments with primates killed by neurotoxic Indian cobra venom that essentially all of the motor nerve cells in the central nervous system were involved by this venom. A basis of Sanders' invention was the discovery that such neurotropic snake venom, in an essentially non-toxic state, also could reach that same broad spectrum of motor nerve cells and block or interfere with invading pathogenic bacteria, viruses or proteins with potentially deleterious functions. Thus, the snake venom used in producing the composition was a neurotoxic venom, i.e., causing

1 death through neuromuscular blockade. As the dosages of venom
2 required to block the nerve cell receptors would have been far more
3 than sufficient to quickly kill the patient, it was imperative that
4 the venom was detoxified. The detoxified but undenatured venom was
5 referred to as being neurotropic. The venom was preferably
6 detoxified in the mildest and most gentle manner. While various
7 detoxification procedures were known then to the art, such as
8 treatment with formaldehyde, fluorescein dyes, ultraviolet light,
9 ozone, heat, it was preferred that gentle oxygenation at relatively
10 low temperatures be practiced, although the particular
11 detoxification procedure was not defined as critical. Sanders
12 employed a modified Boquet detoxification procedure using hydrogen
13 peroxide, outlined below. The acceptability of any particular
14 detoxification procedure was tested by the classical Semliki Forest
15 virus test, as taught by Sanders, U.S. Patent No. 4,162,303.

16 U.S. Pat. No. 3,888,977, issued on June 10, 1975 to Murray J.
17 Sanders (the entire disclosure of which is incorporated herein by
18 reference and relied upon for details of disclosure) teaches that
19 animals, including humans, may be treated for progressive
20 degenerative neurological diseases, such as amyotropic lateral
21 sclerosis, by administration of a modified snake venom neurotoxin
22 derived from the venom of either the Bungarus genus (including the
23 Crotalus genus) or from a combination of the Bungarus genus and the
24 Naja genus, i.e., in either case the therapeutic composition must
25 contain at least in part modified neurotoxin derived from the
26 Bungarus genus. Thus, it is taught that while the Bungarus venom
27 can be effectively used alone, the Naja venom must be used in
28 combination with the Bungarus venom. Unfortunately, however,

1 Bungarus venom is not as readily available as Naja venom; the
2 supply thereof is more uncertain; and it is far more expensive than
3 the Naja venom. Sanders patent 4,126,676 (1978) provided a method
4 of treatment of animals suffering from progressive degenerative
5 neurological diseases wherein the therapeutic modified neurotoxin
6 was derived from the Naja genus alone. Miller, et al. (1977)
7 reported that the modified venoms antiviral activity against
8 Semliki Forest virus was associated with several chromatographic
9 fractions comprising the neurotoxic components. The most abundant
10 component with antiviral activity was shown to be alpha-cobratoxin.
11 Yourist, et al. (1983) reported that modified alpha-cobratoxin
12 could inhibit the activity of herpesvirus. It seemed therefore,
13 that these modified venoms and constituents had significant
14 inhibitory activity against unrelated viruses. This non-specific
15 activity has prompted the examination of these modified venom
16 products against a number of viral types.

17 Other references of interest include four patents, Haast, U.S.
18 Patent Nos. 4,741,902 and 5,723,477, Hoxie, U.S. Patent No.
19 5,994,515 and Au-Yuong, et al., U.S. Patent No. 5,955,303.
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8 SUMMARY OF THE INVENTION

9 The present invention provides a composition and method for
10 treating and preventing retroviral infections of mammalian cells.
11 One aspect of the invention relates to the identification of
12 modified neurotoxins capable of preventing HIV infection and
13 replication in that cell. In another aspect the invention relates
14 to an retroviral composition derived from modified venom which can
15 be administered in-vivo for the treatment of HIV infection. In
16 another aspect, the invention relates to the synergistic effects of
17 modified venom constituents in preventing HIV infection and
18 replication. In another aspect, the retrovirus is selected from
19 the group consisting of Lentiviruses (HIV-1, HIV-2, SIV, EIAV, BIV,
20 and FIV).

21 Proteins such as those from venoms, as described herein, have
22 long been recognized for their ability to bind to specific
23 receptors on the surface of mammalian cells. These neurospecific
24 proteins bind to such common receptors as the acetylcholine
25 receptor for example. However, the protein motif employed by these
26 neurotoxins to affect binding appears to be a common motif employed
27 by other, apparently unrelated, proteins including those present in
28 viral coat proteins. Such viral proteins include rabies virus coat

1 protein and gp120 from HIV. Prior studies had indicated that
2 proteins with these motifs could interfere with the activity of the
3 other. Sanders provided a method which permits the safe
4 administration of venom proteins allowing the application of these
5 laboratory observations to practical use. Therefore included in
6 the invention is a method of treating lentivirus infection in
7 mammals and humans comprising administering to the host of either
8 the modified venom or the modified neurotoxin.

9 In yet another aspect of the invention is the indication that
10 modified neurotoxins can bind to a HIV receptor protein and/or a
11 cellular cofactor unrelated to their original target receptors.
12 The specific entity to which MCTX binds is presently unknown,
13 though it appears to have an impact upon viral infection late in
14 infection, possibly during maturation of infectious particles.

15 In another aspect of the invention, the modified venom's
16 higher antiviral activity suggests the existence of synergism
17 between venom components due to the presence of other neurotoxic
18 components in addition of alpha neurotoxin known as cobratoxin.
19 Therefore, as a group consisting of modified alpha-neurotoxins with
20 homologous domains and acetylcholine receptor binding activity can
21 inhibit lentivirus infection and could be selected from but not
22 limited to alpha-cobratoxin, alpha-bungarotoxin, alpha-cobrotoxin
23 and alpha-conotoxin.

24 25 DETAILED DESCRIPTION OF THE INVENTION

26 Although the survival of individuals currently infected by the
27 HIV virus is dramatically longer than it was 20 years ago, such
28 survival is at the cost of a drug regime which is highly expensive,

1 complicated, relegated to a fixed time and sequence schedule, has
2 adverse physiological side effects and is, ultimately, too little
3 too late. While the logical method to halt the spread of the
4 disease is sexual abstinence, such method embodies so many facets
5 of world society, that, realistically, the disease will remain
6 uncontrollable until such a time as it can be controlled by methods
7 which are inexpensive, have few side effects, and can be
8 administered easily.

9 Prophylaxis, utilized before or after potential exposure,
10 fulfills these requirements. Potential prevention / treatment
11 could take many forms; three are: 1. The development of a vaccine
12 that prevents infection; 2. Prevention of an initial infection or
13 control of the spread of an initial infection that has not
14 progressed to AIDS by a means other than a vaccine, or, 3. A
15 resolution of the syndrome known as AIDS by the use of anti-
16 retroviral agents. While vaccine production is ultimately the most
17 efficacious of the three methods, due to the mutational
18 idiosyncrasies of the virus, such development is not a likely or a
19 probable immediate occurrence. Vaccine development attempts to
20 date have failed to translate into man from animal test-models
21 (Peters; 2000).

22 Medical research resources are currently being applied to the
23 management, rather than the cure of a HIV infection. While the use
24 of anti-retrovirals agents have improved the quality and length of
25 life, they have disadvantages which include toxicity, development
26 of drug resistance, persistence of latently infected cells
27 resulting in viral rebound after prolonged treatment and, finally,
28 high expense. The prevention and/or control of an infection prior

1 to loss of immune capabilities associated with progression to AIDS
2 is currently the most expedient and cost effective method.
3 Currently, there are several approved drugs types that apply
4 themselves to the control of an ongoing HIV infection. These drug
5 types are, nucleoside reverse transcriptase inhibitors, non-
6 nucleoside reverse transcriptase inhibitors and protease
7 inhibitors. These are currently encompassed by highly aggressive
8 anti-retroviral therapy (HAART). All these drug types are
9 susceptible to loss of effectiveness due to genetic mutation of the
10 HIV-1. Thus, the blockade of HIV infection or the control of the
11 spread of HIV infection through the use of fusion or entry
12 inhibitors appears to be the most logical method barring the
13 availability of a vaccine. Such blocking substance, or substances,
14 could be applied topically, as a cream or douche, and provide
15 protection during coitus. The use of this mode of prevention has
16 been suggested by others (Turpin, 2002) and is being implemented
17 (Van Damme, et al., 2000). The utilization of a binding/entry
18 inhibitor as a prophylactic that would block infection and maintain
19 a period of protection in the genital tract could provide an
20 effective measure which would reduce HIV-1 transmission (D'Sousa,
21 et al., 2000). Topical administration would not be amenable to
22 prevention of disease by blood transfer by more direct routes (such
23 as needles). However, as an injectable, or by buccal
24 administration, it could be applicable parenterally in the
25 treatment of an HIV infection during early stages of exposure, or
26 later, by providing control of HIV dissemination within the host.
27 Alternatively, drug activities which alter the virus and
28 reduce its infectivity or alter its functional form upon release

1 would supply a mechanism for infection control at the "other side"
2 of the infection sequence.

3 HIV-1 is a lentivirus (lenti = slow {Latin}) of the family
4 Retroviridae. The virus is enveloped, 80 -130nm in diameter and
5 has an icosahedral capsid. As with other lentiviruses, HIV can
6 infect terminally differentiated, non-dividing cells such as
7 macrophages resident in tissue or brain (microglia) as well as
8 cells of the T cell lineage, specifically CD4+ cells, known as T
9 helper (T_H) cells. Lentiviruses have, through mutation, the
10 capability to infect immune cells (macrophages; T_H -cells), the
11 ability to avoid immune system eradication and, thus, tend to
12 persist for the life of their host. The typical HIV infection
13 progresses through three stages: initial, or acute, associated with
14 high levels of viral replication and dissemination, a latent stage
15 attributed to partial immune system control, which is followed by
16 the third stage which encompasses the return of high levels of
17 viral replication and progression to clinical disease states due to
18 decreased immunocompetence, termed acquired immunodeficiency
19 syndrome (AIDS). HIV is suggested to be derived from the simian
20 immunodeficiency virus (SIV) (Courgnaud, et al., 2001) and first
21 entered the human population between 1915 and 1941 (Korber, et al.,
22 2000). Two HIVs are associated with human AIDS: HIV-1 and HIV-2.
23 HIV-1 is distributed worldwide and is responsible for the current
24 AIDS pandemic while HIV-2 is currently restricted to West Africa.
25 Both are spread by the same routes, though HIV-2 may be less
26 pathogenic.

27 Treatment of HIV infection currently encompasses two basic
28 modalities: drug action at host intracellular targets (post entry)

1 and drug interaction at viral extracellular targets (pre-entry).
2 The latter are termed as binding/entry inhibitors. Extracellular
3 targets are those associated with viral attachment, fusion and
4 entry into the host cell. Intracellular targets are those
5 associated with viral nucleic acid synthesis and processing and are
6 termed as anti-retroviral drugs. There are currently 16 licensed
7 antiretroviral drugs employed to combat HIV-1 infection (D'Souza,
8 et al. 2000, aidsmeds.com, 2002a). Currently, there is a drug, T-
9 20 (Trimeris), which is licensed as a binding/entry inhibitor.
10 Within the context of this proposal, extracellular targets are of
11 immediate importance, consequently, discussions of viral inhibition
12 post-cell entry will be omitted.

13 Infection by HIV occurs following the introduction of the
14 virus to the blood of the potential host. Virus-host cell
15 interaction is mediated through the viral envelope glycoproteins
16 gp120 and gp41 (gp160), which are assembled as trimers on the
17 surface of the viral envelope, and their interactions with host
18 cell surface receptors CD4, and CXCR4 or CCR5. U.S. Patent No.
19 5,994,515 (Hoxie) describes the manner in which the human
20 immunodeficiency viruses HIV-1 and HIV-2 and the closely related
21 simian immunodeficiency viruses (SIV), all use the CD4 molecule as
22 a receptor during infection though viruses like HIV and FIV can
23 infect CD4 negative cells. The latter two host cell surface
24 receptors are chemokine receptors and act as co-receptors along
25 with CD4. Chemokines are a large family of low molecular weight,
26 inducible, secreted, proinflammatory cytokines which are produced
27 by various cell types. See, for instance, Au-Yuong, et al., U.S.
28 Patent No. 5,955,303. Chemokines have been divided into several

1 subfamilies on the basis of the positions of their conserved
2 cysteines. The CC family includes monocyte chemoattractant
3 protein-1 (MCP-1), RANTES (regulated on activation, normal T cell-
4 expressed and secreted), macrophage inflammatory proteins (MIP-
5 1.alpha., MIP-1.beta.), and eotaxin. (Proost, P. (1996) Int. J. Clin.
6 Lab. Res. 26: 211-223; Raport, C. J. (1996) J. Biol. Chem. 271:
7 17161-17166). The CXC family includes interleukin-8 (IL-8), growth
8 regulatory gene, neutrophil-activating peptide-2, and platelet
9 factor 4 (PF-4). Although IL-8 and PF-4 are both polymorphonuclear
10 chemo-attractants, angiogenesis is stimulated by IL-8 and inhibited
11 by PF-4. However, the macrophage tropic (CCR5) strain BaL, is not
12 capable of infecting cells which co-express both CXCR4 and CD4.
13 These results suggest that CXCR4 can serve as a co-factor for T-
14 tropic, but not M-tropic, HIV-1 strains (Feng, et al., 1996,
15 supra). Moreover, the finding that there is a change from M to T-
16 tropic viruses over time in infected individuals correlates with
17 disease progression suggests that the ability of the viral envelope
18 to interact with CXCR4 represents an important feature in the
19 pathogenesis of immunodeficiency and the development of full blown
20 AIDS.

21 There are five variable regions and five conserved regions
22 that compose gp120 (Starcich, et al., 1986; Wyatt, et al., 1995).
23 Two variable loop regions, V1/V2 and V3, prior to initial viral
24 interaction with the cell surface, are closely associated and block
25 accessibility to a region associated with chemokine receptor
26 binding. Binding of CD4, which occurs above these two variable
27 regions, is dependent upon discontinuous elements in conserved
28 regions 3 and 4 (C3 and C4) (Moore, et al., 1994). Amino acid

1 changes in the V2 and V3 loop regions can alter both the membrane
2 fusion process and HIV-1 tropism (Wyatt, et al., 1995).

3 Infection of susceptible cells occurs via three conformational
4 stages involving HIV-1 gp120 (D'Sousa et al., 2000). In short, the
5 interaction between HIV-1 and the host cell proceeds as follows:

6 A segment of gp120 binds to CD4 on the host cell surface resulting
7 in an initial conformational change of the V1/V2 and V3 regions of
8 gp120. This change allows access to a portion of gp120, previously
9 covered by the two variable regions, which binds with a co-receptor
10 resident on the host cell. This gp120 conformational change
11 involves movement of the V1/V2 loops away from the V3 loop (Thali,
12 et al., 1993; Wyatt, et al., 1995, Sullivan, et al., 1998). Under
13 normal circumstances, HIV-1 gp120 requires the presence of both the
14 CD4 and a co-receptor to cause additional conformational changes
15 resulting in exposure of gp41. The viral protein, gp41, is
16 responsible for fusion and entry. The CD4 co-receptor is either
17 CXCR4 or CCR5 and is determined by the tropism of the virus (Feng,
18 et al., 1996; Doranz, et al., 1996; Deng, et al., 1996; Choe, et
19 al., 1996; Wu, et al., 1996). The extracellular portion of gp41
20 contains two helical domains: HR1 and HR2 (or NHR and CHR; Jiang,
21 et al., 2002). The tip of gp41 inserts into the host cell membrane
22 and anchors the virus to the cell. The two helical domains of
23 gp41, previously separated by a segment of gp120, bind together to
24 form a 6-helix bundle that is a fusogenic structure (Jiang, 2002).
25 The virus and cell surface are pulled together by this structure,
26 allowing fusion of the virus envelope and host cellular membrane
27 and insertion of viral genetic material. The co-receptor CCR5,
28 whose natural ligands are the chemokines RANTES, MIP-1- α , MIP-1- β

1 and MDC, is employed by primary isolates of HIV-1 which are
2 generally M (macrophage) tropic, and is found on T cells and
3 macrophages. CXCR4, whose natural ligand is SDF-1a, is employed by
4 late stage HIV-1 isolates and is employed by T (T cell)-tropic HIV-
5 1. There is an in vivo switch in tropism during HIV infection
6 (Wyatt and Sodroski, 1998).

7 Due to the complexity of the binding and penetration of HIV-1,
8 the virus is, at least theoretically, vulnerable to either single
9 or, more especially, multiple entry inhibitors. Therefore, there
10 are several cellular sites and viral sites with which inhibitors
11 could interact to halt the process: CD4, CXCR4, CCR5, gp120 and
12 gp41. The substances currently under consideration generally have
13 high cost in addition to limited production as well as low bio-
14 availability and poor pharmacologic and toxicology profiles.
15 Nineteen potential binding/entry inhibitors were listed in 2000
16 (D'Sousa, et al., 2000); work is still progressing and a glance at
17 the current literature indicates new additions in the list. Gp41
18 inhibitors T-20 and T-1249 (Trimeris/Hoffman LaRoche) as well as
19 PRO-542 (Progenics), PRO-2000 (Procept) and Cyanovirin (CV-N) all
20 of which target virus/CD4 interaction and AMD-3100 (AnorMed), which
21 interferes with HIV/CXCR4 interactions, are still viable
22 candidates. These compounds are representative of, and provide an
23 overview of, current thought in the area of inhibiting viral
24 binding / entry (De Clercq, 2002).

25 The drug candidates listed above suggest that combinatorial
26 efforts to prevent binding and entry is likely to become the norm,
27 as opposed to the use of single drugs, as indicated by the
28 synergistic combination of drugs with T-20. Additionally, the

1 concept of disease prevention by the use of binding / entry
2 inhibitors is established in the research and clinical communities.
3 The use of PRO-2000 in a vaginal gel, coupled with the early
4 results achieved, suggest that this is a potentially viable
5 approach, especially given that this is associated with the most
6 frequent mode of transmission (Greenhead, 2000). This topical
7 approach is strengthened by the determination that HIV must transit
8 the epithelial lining of the vagina wall to access infection
9 susceptible cells, that epithelial cells are not subject to
10 infection and they do not aid transport of the virus. In fact, the
11 epithelial cells may act as a barrier to infection. The presence
12 of PRO 2000 was found to result in 97% reduction in HIV infection
13 in an in-vitro cervical explant test system (Greenhead, 2000).

14
15 Molecular mimicry; alpha-neurotoxin / HIV gp120 sequence homology

16 Death by cobra envenomation is attributed to the interaction
17 of basic polypeptides (cobra alpha-neurotoxins) that act post-
18 synaptically and result in blockade of nerve transmission due to
19 their affinity for the nicotinic acetylcholine receptor (nAChR).
20 nAChRs are ligand-gated ion channels activated by the binding of
21 acetylcholine (Ach). On muscle, the nAChR molecule is a pentamer
22 composed of two alpha subunits, one beta, one gamma and one delta
23 subunit. Ach binds to the alpha subunit, each nAChR complex having
24 two acetylcholine binding sites (Dowding et al., 1987). Cobratoxin
25 and other snake alpha-neurotoxins are curaremimetic since they
26 mimic the actions of curare in that they are potent competitive
27 inhibitors of Ach binding to the nAChR and blocking Ach activity.
28 The action of cobratoxin differs from that of curare and strychnine

1 in that the effects of these two substances in vitro is reversed by
2 washing, while the action of cobratoxin is irreversible. A large
3 number of curaremimetic toxins have been isolated from the venoms
4 of elapid and hydrophid snakes and similar curaremimetic toxins
5 have been isolated from the venom of sea snails of the *Conus*
6 genera. Overall, the snake proteins have a structural homology,
7 being small proteins with a clover leaf-like shape consisting of
8 three adjacent loops that emerge from a small globular core (LeGoas
9 et al., 1992). The neurotoxin of the cobra of interest, *Naja naja*
10 *kaouthia*, is a long chain neurotoxin that is cross-linked with 5
11 disulfide bonds (LeGoas et al., 1992). Loop one is partly
12 hydrophobic and partly exposed to water, this portion having the
13 greater flexibility. The central, or toxic loop, loop 2, is the
14 largest loop and is mainly composed of two strands from the beta-
15 pleated sheet. This loop bears an amino acid sequence homologous
16 with HIV-1 gp120 and rabies virus glycoprotein (RVG). Loop 3 is
17 closed by a disulfide bond and is nearly perpendicular to the beta
18 sheet plane (LeGoas, et al., 1992). All known potent alpha-
19 neurotoxins contain a single invariant tryptophan residue in the
20 same or similar position in the primary sequence (Chang, et al.,
21 1990). This tryptophan residue occupies amino acid position 28 in
22 alpha-bungarotoxin (*Bungarus multicinctus*) and position 25 in
23 alpha-cobratoxin (*Naja naja kaouthia*).

24 The α -neurotoxins of *Naja naja kaouthia* (cobratoxin) and
25 *Bungarus multicinctus* (bungarotoxin) have a sequence homology with
26 HIV gp120 and rabies virus glycoprotein (RVG) as indicated below in
27 Table I. This homology is located in a manner that it is
28 accessible for the production and interaction with antibodies on

both viruses. Like the homologous sequence on elapid toxins, the amino acid sequence present in rabies virus glycoprotein (RVG) and gp120 of HIV results in interaction with the nAChR. This interaction has been demonstrated by the binding of rabies virus (Lentz, et al., 1982, Lentz, et al., 1987) and HIV-1 gp120 (Bracci, et al., 1992). Both viral interactions were blocked by the use of -bungarotoxin.

The apparent domain of sequence homology on HIV gp120 is located at amino acid residues 159 -169, which places it at the initiation of the loop of the gp120 variable region 2 (V2), and is associated with the V1/V2 loop region.

TABLE I: SEQUENCE HOMOLOGY OF HIV-1, RVG and SNAKE NEUROTOXINS

RVG (189-199)		C	D	I	F	T	N	S	R	G	K	I					
HIV-gp120 (159-169)								F	N	I	S	T	S	I	R	G	K
		V															
Peptide B2				S	F	N	I	S	T	S	I	R	G	K	V	Q	
	I																
-cobratoxin (30-40)						C	D	A	F	C	S	I	R	G	K		
		R															
-bungarotoxin (30-40)				C	D	A	F	C	S	S	R	G	K				

From Neri et.al.; 1990; Bracci et. al.; 1992; Bracci et. al.; 1997, Meyers and Lu; 2002

The apparent domain of sequence homology on HIV gp120 is

1 located at amino acid residues 159-169, which places it at the
2 initiation of the loop of the gp120 variable region 2 (V2), and is
3 associated with the V1/V2 loop region.

4 There are five variable regions and five conserved regions on
5 gp120 (Starcich, et al., 1986; Wyatt, et al., 1995). Binding of
6 CD4 is dependent upon discontinuous elements in conserved regions
7 3 and 4 (C3 and C4) while the V3 and V4 regions are the most
8 exposed elements of the multimeric envelope glycoprotein complex
9 (Moore, et al., 1994). Changes in the V2 and V3 loop regions can
10 alter both the membrane fusion process and HIV-1 tropism (Wyatt, et
11 al., 1995).

12 The sequence homology existing between gp120 and snake a-
13 neurotoxins is not obviously associated with the host cell CD4
14 binding, in the context of a known receptor sequence on the CD4
15 molecule. Thus there does not appear to be an obvious association
16 between the sequence and viral interaction with potential host
17 cells, given the currently accepted binding / entry scenario. With
18 respect to that scenario, as indicated previously, there are
19 considerable viral conformational alterations associated with the
20 CD4-gp120 interaction. Binding thermodynamics, as reported by
21 Myszka, et al; (2000), are of unexpected magnitude and indicative
22 of extensive structural rearrangements. One of these
23 rearrangements is the movement of the V1/V2 loops which results in
24 the exposure of the conserved discontinuous structures which are
25 recognized by monoclonal antibodies (Thali, et al., 1993; Wyatt, et
26 al., 1995; Sullivan, et al., 1998). Conformational alterations of
27 the V1/V2 loop structures also result in exposure of the site for
28 interaction with the CCR5 chemokine receptor (Kolchinsky, et al.,

2001). It has been suggested, based upon an induced mutation of the $\alpha 3$ strand of the bridging sheet between V1/V2 and V3 (Zhu, et al., 2001), that there is a direct interaction between V1/V2 and V3. Since the V2 loop gp120 site is exposed on an aspect of the protein that interacts with the potential host cell (Wyatt and Sodroski, 1998), and the demonstrable presence of nAChR on CD4+ cells, there is a possibility that a natural reaction with HIV-1 with nAChR occurs. The ability of HIV-gp120 to bind to the nAChR as well as the proven capability of modified neurotoxin to bind to the same receptor permits the hypothesis that modified neurotoxins may act as an entry inhibitor particularly in the nervous system.

The presence of nAChR on CD4+ cells.

A better and more documented rationale for modified neurotoxins' potential as HIV-1 entry inhibitors of lymphocytes is in the interaction of the homologous α -neurotoxins with nAChR present on CD4+ cell surfaces. Human "T" lymphocytes are a major source for acetylcholine (ACh) (Fujii and Kawashima, 2001; Sato, et al., 1999; Kawashima, et al., 1998; Fujii, et al., 1996). Additionally, there is a substantial body of work indicating the presence of both muscarinic AChRs (mAChRs) and nicotinic AChRs on the surface of human peripheral blood mononuclear cells (PBMC) (Fujii and Kawashima, 2001; Singh, et al., 2000; Kawashima and Fujii, 2000). Messenger RNA expression of subunits for both nAChR ($\alpha 2 - \alpha 7$ and $\alpha 2 - \alpha 4$) and mAChR ($m1 - m5$) was determined for human PBMC indicating the presence of AChR on the cell surface (Sato, et al., 1999). Others (Battaglioli, et al., 1998) have determined the

1 presence of the nAChR $\alpha 3$ promoter in T lymphocytes. Stimulation of
2 T lymphocytes with the mitogen phytohemagglutinin (PHA) results in
3 increased synthesis and release of Ach as well as an increase in
4 mRNA encoding for nAChR and mAChR (Kawashima and Fujii, 2000; Fujii
5 and Kawashima, 2001) and suggests an autocrine and / or paracrine
6 function for Ach in the regulation of immune function (Fujii and
7 Kawashima, 2001). Inhibition of Concanavalin-A (Con A) induced T
8 cell proliferation is blocked by the nAChR antagonist mecamylamine
9 (MEC) and by acute nicotine exposure (Singh et al., 2000). Acute
10 nicotine exposure of ConA stimulated mouse splenocytes resulted in
11 decreased production of IL-10 and also resulted in increased
12 production of IFN-gamma (Hallquist, et al., 2000). The presence of
13 human lymphocyte cell surface nAChRs has been determined by the
14 binding of fluoresceine isothiocyanate (FITC)-conjugated α -BTX;
15 affinity purification of α -BTX bound protein indicated that the
16 nAChR bound were the same as those found in muscle (Toyabe et al.,
17 1997). Additionally, a monoclonal antibody (MoAb), designated as
18 W6, competes with Ach for binding with α -BTX for the Torpedo nAChR
19 $\alpha 1$ subunit (McLane et al., 1992). MoAb W6 mediated immuno-staining
20 indicated the presence of nAChR on the surface of human PBMC which
21 was situated in the perinuclear / surface region and which
22 resembled the binding of antibody specific for CD4+ (Hiemke et al.,
23 1996). The presence of surface $\alpha 3$ and $\alpha 4$ nAChR subunits was
24 determined on human PBMC (Hiemke, et al., 1996) and studies by
25 Benhammou, et al. (2000) using nicotine binding and determination
26 of mRNA expression in PBMC also indicated the presence of $\alpha 4$ - $\alpha 3$ and
27 $\alpha 3$ - $\alpha 4$ nAChRs. Others have determined the binding of ^3H -nicotine to

1 human PBMC indicating the presence of nAChR on the surface with a
2 calculated density of ~2000 sites/cell (Grabczewska, et al., 1990).
3 Additionally the binding of ³H-nicotine to human neutrophils,
4 monocytes and lymphocytes (Davies, et al., 1982) has been observed.
5 The formation of E- rosettes, a function of T cells from peripheral
6 blood, and a method used for T cell enumeration, is decreased by
7 30% - 40% in the presence of carbamylcholine chloride, a
8 cholinergic antagonist, indicating the expression of nAChR on at
9 least a subset of human T cells (Mizuno, et al., 1982).

10 Therefore the target receptor for venom alpha-neurotoxins are
11 readily expressed in a variety of cells that can also be infected
12 with HIV. However, studies with other viruses have shown that
13 native alpha-cobratoxin does not have any antiviral activity
14 against either herpes or Semliki Forest virus. Formalin or heat
15 denatured venom or cobratoxin, respectively, also displayed no
16 antiviral activity while the heat-denatured CTX (resulting in beta
17 elimination at the disulphide bonds as measured by mass
18 spectrometry) was still capable of binding to its native receptor.
19 Also, inhibition of viral infection, as by the rabies virus, could
20 be observed in cells devoid of NACHR (BHK-21). Therefore it seemed
21 unlikely that the NACHR receptor was part of the antiviral
22 mechanism. Thus, the type of chemical modification is important to
23 the activity of the final product.

24 25 Production Techniques

26 Administration of a highly toxic substance such as cobratoxin
27 for therapeutic purposes is fraught with obvious difficulties, even
28 when highly diluted. As a diluted substance, its potential

effectiveness is reduced, and due to its high affinity for the nAChR, continued use could result in accumulation of the toxin at neuromuscular junctions and the diaphragm with the potential for adverse events. Alpha cobratoxin, of the Thailand cobra, *Naja naja kaouthia*, is a homogeneous non-glycosylated polypeptide composed of 71 amino acids with a molecular weight of 7821d and a pI of 9.6. Detoxification of alpha-cobratoxin can be achieved by exposure to heat, formamide, hydrogen peroxide, perchloric acid, ozone or other oxidizing agents. The result of exposure of cobratoxin to oxidizing agents is modification of amino acid side chains as well as the lysis of one or more disulfide bonds. Tu (1973) has indicated that the curaremimetic alpha-neurotoxins of cobra and krait venoms lose their toxicity upon either oxidation or upon reduction and alkylation of the disulfide bonds. The procedures used for detoxification described here are based upon the work of Sanders, who preferred the use of hydrogen peroxide (Sanders, et al., 1975). Loss of toxicity by oxidized alpha-neurotoxins (MCTX), as cobratoxin, can be determined by the intraperitoneal (IP) injection of excess levels of the modified protein into mice. In general, injection of 1.5 mcg of natural cobratoxin will result in the death of a 25g mouse within 25 minutes. After detoxification, IP injection of a 200mcL volume of 10mg MCT/mL is non-toxic. This represents at least a 1300 fold reduction of toxicity.

Alternatively, an enzyme linked immunosorbant assay (ELISA) can evaluate loss of toxicity, as well as potential potency in terms of continued ability to bind to the nAChR. Although detoxified cobratoxin has lost a considerable proportion of its affinity for the nAChR, sufficient affinity remains such that it

1 can be detected by an ELISA. This enables a measurement of the
2 depression in binding of the modified neurotoxin to nAChR,
3 indicative of loss of toxicity, while simultaneously indicating a
4 continued ability of the modified toxin to bind to the nAChR
5 providing a measure of potency (Raymond; unpublished data). To
6 test the effectiveness, or potency of detoxified venom, Sanders
7 utilized a plaque assay with Semliki Forest virus (Miller, et al.,
8 1977).

9 Sanders applied detoxified cobra venoms to the treatment
10 of polio (Sanders, et al., 1953, 1954a, 1954b, 1958a, 1958b) in
11 primates and amyotrophic lateral sclerosis (ALS) (Sanders and
12 Fellows 1974, 1975, 1978) over a 14-year period under an FDA
13 approved IND. Sanders based his work around the observations of
14 Lamb and Hunter (1904) who demonstrated central nerve cell
15 destruction following *Naja naja* venom exposure. Sanders postulated
16 the notion of steric interference and /or molecular mimicry where
17 detoxified neurotoxins would have the similar access to the CNS and
18 be capable of blocking nerve cell receptors rendering them
19 unavailable for involvement by deleterious neuro-invasive bacteria,
20 viruses or proteins. Thus, the progression of degenerative
21 neurological diseases could be halted or their progression slowed
22 allowing the immune system time to resolve the disease state.

23 In a preferred embodiment, the method of the present
24 invention is used to prepare inactivated forms of venoms or
25 neurotoxins, and more preferably neurotoxins listed in the group
26 below.

Snake Venoms	Naja sp., Bungarus sp., Ophiophagus sp., Hemachatus sp., Boulengeria sp., Pseudohaje sp., Walterinnesia sp., Dendroaspis sp., Elaps sp., Acanthophis sp., Notechis sp., Oxyuranus sp., Pseudechis sp., Pseudonaja sp., Aipysurus sp., Astrotia sp., Enhydrina sp., Hydrophis sp., Lapemis sp., Laticauda sp., Pelamis sp.,
Other venom	Conus sp.
a-Neurotoxins	-cobratoxin, -cobrotoxin, -bungarotoxin, erabutoxin, -conotoxins and muscarinic anticholinergic proteins, M1, M2 and M3.

Recombinant techniques may prove useful in the production of this antiviral peptides. The cloning of a variety of neurotoxins have proven successful though the majority of efforts have focused upon those toxins which are found only in low quantities in native venoms (Fiordalisi, et al., (1996) Toxicon 34, 2, 213-224, Krajewski, et al. (1999) "Recombinant m1-toxin" presented at the 29th Annual Meeting of the Society for Neuroscience) and also with the desire to produce mutants to study structure/function relationships (Smith, et al., (1997) Biochemistry, 36, no. 25, 7690-7996. Cobratoxin has been cloned (Antil S., Servent D. and Menez A. J. Biol. Chem. (1999) Dec 3;274(49):34851-8)[.] Although cobratoxin is abundant and easily obtained from natural sources, in order to study the effect of mutations on its interactions with the acetylcholine receptor, specific recombinant production is desirable. Several bioengineered variants have been proposed by

1 the author who was a contributor to the Smith, et al. (1997) paper
2 which replace the residues required for disulphide bond formation
3 with other residues so as to closely mimic the effects of chemical
4 modifications. As these amino acid substitutions must be expressed
5 in-vivo the availability of modifications are limited to the use of
6 native residues (the standard 20 naturally occurring amino acids)
7 and the host to be employed for expression. In the host the codon
8 usage will be important in ensuring efficient and maximal
9 expression of the novel protein. Theoretically any amino acid can
10 be substituted for cysteine but as this is a more costly approach
11 to generating cobratoxin variants relative to synthetic peptide
12 techniques certain residues have been selected which best reproduce
13 the protein characteristics resulting from chemical exposure. It
14 is usual in this circumstance to make what are considered to be
15 conservative substitutions. As a result, it has been chosen to
16 initially limit the cysteine replacement to the following residues;
17 methionine (M), glutamic acid (E), aspartic acid (D), glutamine
18 (Q), asparagine (N), serine (S), glycine (G) and alanine (A).
19 Methionine incorporation would could be considered to be the more
20 conservative substitution by replacing one sulphur-containing
21 residue for another. Unlike cysteine, methionine cannot form
22 disulphide bonds. Methionine also reacts readily with oxidizing
23 agents to produce the sulfone derivative therefore the purified
24 product can be exposed to chemical agents to confer upon the
25 protein other desirable properties (i.e., low immunogenicity).
26 Also the presence of methionine also allows for the cleavage of the
27 protein into fragments employing cyanogen bromide. Cleavage of the
28 native cobratoxin and modified protein is easily achieved with

1 serine proteases (i.e., trypsin) but at sites containing positive
2 residues. This permits also the evaluation and production of
3 smaller peptide fragments for biological activity (Hinmann, et al.,
4 1999). The conversion of cysteine to cysteic acid also argues for
5 the substitution by other acidic residues such as E, D, Q, N and S.
6 The substitution of E and D for cysteine is estimated to produce a
7 protein with a pI similar to that of modified cobratoxin (pI =
8 4.5). The substitution of cysteine with the residues glycine and
9 alanine would represent standard "neutral" substitutions. The
10 method for creating these genes has been described previously
11 (Smith, et al., 1997). The codon usage of the DNA fragments is
12 optimized for use in commercially used bacterial and yeast
13 expression systems *Escherichia coli* and *Pichia pastoris*
14 respectively.

15 Current technology has also allowed for the production
16 neurotoxins through peptide synthesis. Many smaller neurotoxins
17 (from conus snails, bee venom and scorpion venom) are routinely
18 produced by synthetic peptide methodology (Hopkins, et al., (1995)
19 *J. Biol. Chem.*, 270, no. 38, 22361-22367, Ashcom and Stiles,
20 (1997) *Biochem. J.* 328, 245-250, Granier, et al., (1978) *Eur. J.*
21 *Biochem.*, 82, 293-299 and Sabatier, et al., (1994) *Int. J. Pept.*
22 *Protein Res.*, 43, 486-495) and some are available from commercial
23 organizations. The above references also describe the synthesis of
24 such peptides incorporating mutant residues (Hopkins, et al.
25 (1995) and Sabatier, et al (1994)). Current techniques in peptide
26 chemistry allow for proteins in excess of 80 amino acids can be
27 reliably produced using automated Fmoc solid phase synthesis (ABI
28 433A Peptide Synthesizer, Perkin Elmer - see www.perkin-elmer.com).

1 Non-native amino acids (acetamidomethyl cysteine,
2 carboxyamidomethyl cysteine, cysteic acid, kynurenine and
3 methionine sulphone) can be acquired from Advanced Chemtech
4 (Louisville, Kentucky) or Quchem (Belfast, Ireland). Other
5 oxidized or alkylated amino acid variants are available from these
6 agents. The generation of a synthetic version of the neurotoxin
7 can be achieved by substituting primarily the cysteine residues
8 (from 1 pair to all 5 disulphide couples) with those residues
9 described above to mimic the effects of the various chemical
10 modifications. Furthermore the substitution of other native and
11 non-native residues for cysteine can be investigated in an attempt
12 to identify neurotoxin variants with improved biological activity.
13 Also peptide fragments from within the cobratoxin sequence can be
14 created (analogous to Hinmann et al., (1999), Immunopharmacol.
15 Immunotoxicol., 21 (3), 483-506) and examined for receptor binding
16 activity.

17 To inhibit infection of cells by HIV in vitro, cells are
18 treated with the MCTX of the invention, or a derivative thereof,
19 either prior to or concurrently with the addition of virus.
20 Inhibition of infection of the cells by the MCTX of the invention
21 is assessed by measuring the replication of virus in the cells, by
22 identifying the presence of viral nucleic acids and/or proteins in
23 the cells, for example, by performing PCR, Southern, Northern or
24 Western blotting analyses, reverse transcriptase (RT) assays, or by
25 immunofluorescence or other viral protein detection procedures.
26 The amount of MCTX and virus to be added to the cells will be
27 apparent to one skilled in the art from the teaching provided
28 herein.

1 To inhibit infection of cells by HIV in vivo, the MCTX of the
2 invention, or a derivative thereof, is administered to a human
3 subject who is either at risk of acquiring HIV infection, or who is
4 already infected with HIV. Prior to administration, the MCTX, or
5 a derivative thereof, is suspended in a pharmaceutically acceptable
6 formulation such as a saline solution or other physiologically
7 acceptable solution which is suitable for the chosen route of
8 administration and which will be readily apparent to those skilled
9 in the art of MCTX preparation and administration.

10 Typically, the MCTX is administered in a range of 0.1 mcg to
11 2 mg of protein per dose. Approximately 1-10 doses are
12 administered to the individual at intervals ranging from once per
13 day to once every few years. The MCTX may be administered by any
14 number of routes including, but not limited to, subcutaneous,
15 intramuscular, oral, intravenous, intradermal, intranasal or
16 intravaginal routes of administration. The MCTX of the invention
17 may be administered to the patient in a sustained release
18 formulation using a biodegradable biocompatible polymer, or by on-
19 site delivery using micelles, gels and liposomes, or rectally
20 (e.g., by suppository or enema). The appropriate pharmaceutically
21 acceptable carrier will be evident to those skilled in the art and
22 will depend in large part upon the route of administration.

23 24 EXAMPLES

25 Example 1

26 Venom modification

27 Venom from the Thailand cobra (*Naja naja kaouthia*) was purchased
28 from Biotoxins (Florida) or Kentucky Reptile Zoo (Kentucky).

1 Employing the procedure described by Sanders (Pat. No. 3,888,977)
2 and Miller, et al. (1977) the reactive molecule, hydrogen peroxide,
3 the precursor protein is modified through the addition of oxygen
4 molecules.

5 Other venoms detoxified in this manner include venoms from
6 *Naja naja atra*, *Bungarus multicinctus*, and *Crotalus durissus*
7 *terrificus*.

8 9 Example 2

10 Neurotoxin modification

11 Cobratoxin (CTX) has a molecular weight of 7821 and is
12 composed of 71 amino acids. Alpha-cobratoxin from the Thailand
13 cobra (*Naja naja kaouthia*) was purchased from Biotoxins,
14 Kississimi, Florida. Employing the procedure described by Sanders
15 (Pat. No. 3,888,977) and Miller, et al. (1977) the reactive
16 molecule, hydrogen peroxide, the precursor protein is modified
17 through the addition of oxygen molecules.

18 A modified neurotoxin (MCTX) solution has an acidic pH and a
19 pI of approximately 4.5. Cobratoxin solutions are basic having pH
20 of [10.4]. 8.5. In solution, the drug migrates through molecular
21 sieving gels as monomers, dimers and tetramers. Cobratoxin
22 migrates under these conditions as a monomer. Upon analysis on
23 NuPAGE (Stratagene) SDS polyacrylamide gel electrophoresis (PAGE)
24 the cobratoxin migrates as a 14Kd and 8Kd protein with a reference
25 to comparable proteins under unreduced and reduced conditions
26 respectively. MCTX migrates under reduced and unreduced conditions
27 without change. A single protein band is not obtained showing a
28 diffuse smear from the loading gel down to a molecular weight

1 equivalent to 8Kd. Additionally, the protein is resistant to
2 staining with standard coomassie dyes. By ion exchange, cobratoxin
3 and MCTX have generally opposite properties consistent with the
4 proteins' charges. Specialized ion-exchange chromatographic resins
5 and conditions can be employed to confirm the retention of positive
6 charges which are considered critical for neuroactive properties.

7 As defined by mass spectrometry the molecular weight of MCTX
8 both purified and in venom is 6,777 to 8,000 daltons. Smaller than
9 expected molecular weights suggest protein fragmentation or side
10 chain modifications. Smaller than expected molecular weights
11 suggest protein fragmentation. Current analytical techniques allow
12 for limited structural identification of the number and location of
13 oxidized residues being added to the protein and rely heavily on
14 previously published information and current chemical theory.
15 Amino acid analyzers do not recognize unnatural amino acids and
16 have limited capabilities for this application.

17 18 Example 3

19 Toxicity assay in mice

20 The endpoint of the above reactions are most easily determined
21 by assessing the toxicity of the preparation in mice. Mice are
22 sensitive to the actions of many venoms particularly to that of
23 snakes. The proven LD50 of pure alpha-cobratoxin in mice is 1.2
24 mcg with death observable within hours when injected subcutaneously
25 or intraperitoneally. If the animal survives overnight it is
26 accepted that the material is not lethal and defines the endpoint
27 of the assay. By administering the composition of the invention at
28 set periods a reduction in the material's toxicity can be observed

1 as an increase in time to death. When 5 mg of the protein solution
2 can be administered without inducing death then the reaction
3 process is complete. This represents more than a 4000 fold
4 reduction in toxicity. It is at this point that the solution takes
5 on its antiviral properties and native cobratoxin does not
6 demonstrate antiviral activity in similar assays.

7 8 Examples 4

9 Antiviral Experiments with modified venom and neurotoxin.

10 Based upon findings that modified snake alpha-neurotoxins have
11 lymphocyte chemotactic functions, as well as an observed amino acid
12 sequence homology between HIV-1 gp120 and cobratoxin, the ability
13 of oxidized venom and the purified alpha-cobratoxin to block in
14 vitro HIV-1 infection in a thymus explant system and in PHA
15 stimulated PBMC was examined. PHA stimulated PBMC were infected
16 with a TCID₅₀ of 200 and 1000 of virus (R5 isolate HIV-1_{Ba1} or X4
17 isolate HIV-1_{Lai}).

18 Both formulations demonstrate inhibition of the virus.
19 However, the crude venom preparation unexpectedly demonstrated a
20 higher inhibitory activity than that of the purified neurotoxin.

21 As a generalized procedure for the two laboratories involved
22 in the in vitro testing of oxidized purified alpha neurotoxin and
23 oxidized venom, the following was performed: PBMC from fresh, HIV-1
24 non-infected buffy coat cells obtained from healthy donors at local
25 blood banks were purified by the Ficoll method. The buffy coat
26 cells were maintained at room temperature until centrifugation.
27 Purified PBMC were re-suspended at 1E6 - 3E6 cells / mL RPMI medium
28 supplemented with 10% human AB serum and immediately treated with

1 5ug PHA / mL suspension. Two to three days later, cells were
2 counted and used for examination of infection. As a standard
3 procedure, cells were incubated in propagation media, consisting of
4 RPMI media supplemented with 10% human AB serum and 50 units IL2 /
5 mL, at a density of 6E6 cells per mL and incubated with 200 - 1000
6 TCID₅₀ HIV-1 / mL X 10E6 PBMC. Infection was allowed for 2 hours
7 at 37°C and the unbound virus was washed away by two washes with
8 propagation media. 200,000 cells were suspended in 180uL of
9 propagation media and placed in 96 well plates (U bottom). Twenty
10 uL of a 10X stock of the corresponding dilution of the drug was
11 added to each well. Infections were performed in triplicate and
12 controls containing 1uM AZT were run in parallel as controls to
13 confirm the validity of the assay. The cultures were incubated at
14 37°C for 4 days. At that time, 90uL of media was removed and
15 replaced with 100uL of propagation media containing the
16 corresponding dilution of drug. The amount of p24 accumulated in
17 the culture was estimated 3 days later (7 days post infection) with
18 a Becton-Dickenson p24 ELISA. Routinely, a few samples were chosen
19 and 10E-2 to 10E-4 dilutions of culture supernatant were prepared
20 to estimate the linearity of the assay.

21 22 23 Example 5

24 Preliminary Studies in Patients with HIV by parenteral
25 administration.

26 Based upon the broad antiviral activity of the modified cobra
27 venoms and the purified alpha-cobratoxin concomitant with the
28 proven safety data in prior human trials a preliminary study was

1 undertaken.

2 Twenty (20) HIV positive patients volunteered to undergo
3 treatment with the oxidized alpha-cobratoxin in addition to ten
4 (10) HIV negative individuals over a period of 6 months. The
5 modified cobratoxin was their sole therapy regime. Given the
6 severity of the disease in this patient cohort no HIV positive
7 placebo was examined. The drug was administered initially at 1mcg
8 per day (drug format was 10mcg/ml in 0.9% saline) increasing daily
9 in 0.1cc increments to 10mcg/day and subsequently rising to
10 20mcg/day (administered as 1cc b.i.d.). The participants were
11 supplied with insulin-type syringes and taught to self-administer
12 the drug. The participants presented themselves regularly for
13 blood draws. Full blood analysis was undertaken and the data
14 recorded.

15 No adverse events were reported in normal patients. General
16 responses in HIV positive patients were good with one reported
17 adverse event in a French female aged approximately 28 who was
18 unavailable for follow-up investigations. Notable observations
19 within 2-3 weeks of treatment were improved energy and strength,
20 improved appetite and cessation of diarrhea episodes. Several
21 patients were noted to have increased in weight by over 15 pounds.
22 General activity increased with several patients returning to full
23 employment.

24 The T4/T8 ratios were recorded and reported in figure 1. In
25 normal individuals the ratio is ~1. The curves presented represent
26 a least squares linear regression of the available data for each
27 individual over the period of testing for that individual. Overall
28 the general trend of the ratios was to increase over the course of

1 treatment in the majority of HIV-1 positive patients.

2
3
4 Example 6

5 Preliminary Studies in Patients with HIV by oral
6 administration.

7 Seven individuals self-administered the MCTX using a buccal
8 spray composed of 600mcg/mL saline. The protocol provided for the
9 administration of the drug at 0.1ml seven times per day giving a
10 maximum drug level of 0.7ml (600mcg/ml) x 50% (efficiency of oral
11 delivery) = 210mcg per day over the course of 3 months. Data
12 obtained for this study suggest MCTX had a noticeable effect in
13 three areas: The percentage of HIV-1 infected T cells, the
14 percentage of HIV-1 infected monocytes and percentage Plasma Viral
15 Load. In all three cases, there was a general trend in the
16 majority of patients toward a decrease in infected cells and plasma
17 viral load, some by as much as 40%.